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Coculturing Human Islets with Proangiogenic Support Cells to Improve Islet Revascularization at the Subcutaneous Transplantation Site

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While subcutaneous tissue has been proposed as a clinically relevant site for pancreatic islet transplantation, a major issue of concern remains, which is its poor vascular state. In an effort to overcome this limitation, we present an efficient and reproducible method to form human composite islets (CIs) with proangiogenic cell types in a controlled manner using nonadherent agarose microwell templates. In this study, we assessed the three-dimensional structure, function, and angiogenic potential of human CIs with human mesenchymal stromal cells (hMSCs), with or without human umbilical vein endothelial cells (HUVECs), and preconditioned hMSCs (PC-hMSCs) in EGM-2 under shear stress. Distinct cellular rearrangements could be observed in CIs, but islet functionality was maintained. *In vitro* angiogenesis assays found significantly enhanced sprout formation in case of CIs. In particular, the number of sprouts emanating from CIs with PC-hMSCs was significantly increased compared to other conditions. Subsequent *in vivo* assessment confirmed the proangiogenic potential of CIs. However, in contrast to our *in vitro* angiogenesis assays, CIs with hMSCs and HUVECs exhibited a higher *in vivo* angiogenic potential compared to control islets or islets combined with hMSCs or PC-hMSCs. These findings highlight the importance and necessity of verifying *in vitro* studies with *in vivo* models to reliably predict, in this case, revascularization outcomes. Regardless, we demonstrate here the therapeutic potential of CIs with proangiogenic support cells to enhance islet revascularization at a clinically relevant, although poorly vascularized, transplantation site.

Introduction

Clinical trials have demonstrated the ability of allogeneic islet transplants to regulate blood glucose levels in patients with type 1 diabetes and labile glycemic control.[1–3] The main benefit of this procedure compared to whole-organ transplantation is the significant reduction in glycemic fluctuations while having reduced postoperative trauma and complication rates. In clinical practice, the transplantation site of choice is the liver. However, the long-term insulin independence rate at this site is disappointing due to substantial islet loss, necessitating the use of at least two donor organs to cure one patient.[1,3] There is strong evidence that site-specific factors contribute to this islet loss in the liver, such as the exposure to high concentrations of immunosuppressants[4] and the instant blood-mediated inflammatory reaction.[5,6] This has led to the search for alternative transplantation sites.

The subcutaneous space is a relevant candidate for islet transplantation because the transplant and biopsy procedures for this site are simple with minimal invasion. Furthermore, this site holds the capacity to transplant a sufficient amount of islets. However, a major challenge of this site is its poor vascularization state.[7] Since the vascular network is

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important to maintain the islets' oxygen-dependent metabolism and their ability to quickly secrete insulin in response to changes in blood glucose levels, a reduced vascularization will affect both islet survival and function. Therefore, the vascular connections need to be re-established as fast as possible after transplantation.

Hence, various attempts have been made to improve vascularization of islets after transplantation. One of these attempts is to increase the action of proangiogenic factors to stimulate the proliferation, migration, and maturation of endothelial cells.[8] Precise control over timing, dose delivery, and effect duration of these factors remains a major challenge to obtain mature functional blood vessels within the islets. An alternative approach is to directly use endothelial cells, endothelial progenitor cells, or mesenchymal stem cells (MSCs). Johansson *et al.*[9] have shown that coating human islets with endothelial cells initiates the formation of vessel-like structures *in vitro*, without impairing islet functionality. The sprouting capacity of endothelial cell-coated islets was further improved by the addition of MSCs. Other studies have shown that cotransplanting islets with mature endothelial cells,[10] stromal cells,[11–15] or endothelial progenitor cells[16–18] derived from various sources can induce neovascularization, resulting in enhanced islet revascularization and better function, regardless of whether the implantation site was underneath the kidney capsule[10,11,14,16,18,19] or the liver.[12,15,17] However, a comparison of islet revascularization at the subcutaneous transplantation site when using different support cell types has not been performed.

In the current study, we report an efficient and reproducible method to form human CIs with proangiogenic cell types in a controlled manner using nonadherent agarose microwell templates. Recently, we have shown that preconditioning of human mesenchymal stromal cells (hMSCs) in EGM-2 under shear stress, factors known to play an important role in triggering endothelial differentiation of MSCs,[20–23] significantly improves the vascularization of Dex-g-HA gels after subcutaneous implantation.[24] Therefore, in this study, we directly compare the angiogenic potential of composite islets (CIs) formed with a mixture of hMSCs and human umbilical vein endothelial cells (HUVECs), hMSCs, or preconditioned hMSCs (PC-hMSCs) to that of control islets at the subcutaneous transplantation site.

Materials and Methods

Islets of Langerhans isolation and culture

Human islets of Langerhans were isolated from pancreas obtained from organ donors (Human Islet Isolation Laboratory, Leiden University Medical Center, Leiden, The Netherlands). Islets were used in the studies if they could not be used for clinical transplantation, and if research consent was available, according to national laws. Islets were cultured in islet culture medium (CMRL-1066; Cellgro, Mediatech, VA), supplemented with 10% fetal bovine serum (FBS) (Lonza, Verviers, Belgium), 100 U/mL penicillin (GIBCO, Bleiswijk, The Netherlands), and 10 μg/mL streptomycin (GIBCO).

hMSC isolation and culture

Bone marrow aspirates were obtained from donors with written informed consent (Medisch Spectrum Twente, Enschede, The Netherlands), and hMSCs were isolated and proliferated as described previously.[24] Briefly, aspirates were resuspended using a 20-G needle and plated at a density of 0.5 million mononucleated cells per cm^2. Cells were grown in MSC proliferation medium (alpha-MEM; GIBCO), supplemented with 10% FBS (Lonza), 100 U/mL penicillin (GIBCO), 10 μg/mL streptomycin (GIBCO), 2 mM L-glutamine (GIBCO), 0.2 mM L-ascorbic acid 2-phosphate magnesium salt (Sigma-Aldrich, Diegem, Belgium), and 1 ng/mL basic fibroblast growth factor (bFGF) (Fisher Scientific, Waltham, MA) at 37°C in a humid atmosphere with 5% CO_2. Cells were expanded up to passage 2. For further experiments, hMSCs from different donors and one immortalized clone (iMSCs, courtesy of Prof. Ola Myklebost, University of Oslo, Norway)[25] were cultured in basic medium (alpha-MEM supplemented with 10% FBS, 100 U/mL penicillin, 10 μg/mL streptomycin, 2 mM L-glutamine, and 0.2 mM ASAP). HUVECs (Lonza) were cultured in endothelial growth medium (EGM-2; Lonza).

Preconditioning of hMSCs

For preconditioning, iMSCs (passage 25, for *in vitro* sprouting assay) and hMSCs from different donors (passages 1–2) were seeded at a density of 3000 cells per cm^2 on tissue culture plastic in EGM-2 and cultured for 10 days. Next to growth supplements, shear force has also been shown to play an important role in triggering endothelial differentiation of MSCs.[20–23] Therefore, we applied shear force after 1 day of static culture using an orbital shaker (20 rpm), as described previously.[24] Cells that were cultured according to this protocol will be referred to as PC-hMSCs.

Cell labeling

When indicated, hMSCs (both naive and preconditioned) and HUVECs were labeled using CM-DiI (red) or CM-DiO (green), according to the manufacturer's protocol (Life Technologies, Bleiswijk, The Netherlands).

Formation of CIs

Nonadherent agarose microwell chips were prepared by replica molding as described previously.[26,27] Briefly, negative replicates of patterned polydimethylsiloxane (PDMS; Sylgard 184, Dow Corning, Midland) stamps, each containing 130 pillars with a diameter of 400 μm and a height of 200 μm, were prepared using 3% agarose solution (UltraPure Agarose; Invitrogen, Bleiswijk, The Netherlands). Before cell seeding, the agarose chips were incubated in EGM-2 for 8 h. To produce CIs with proangiogenic cells, islets were homogeneously seeded in the microwells, after which a cell suspension of hMSCs (1250 cells/islet), a mixture of hMSCs (625 cells/islet) and HUVECs (1250 cells/islet), or PC-hMSCs (1250 cells/islet), was added. Subsequently, the agarose microwell chips were shortly centrifuged at 150 *g* to settle the cells with the islets in the microwells. Cells attached to the surface of the islets within 8 h of culture in EGM-2. These cell aggregates are referred to as CIs. Medium was refreshed every day with a 1:1 mixture of EGM-2 and islet culture medium. Imaging of the CIs was performed using an EVOS® FL Imaging System (Invitrogen).

Immunohistochemistry on whole-mount islets

At day 1 and day 5, CIs and control islets were flushed out of the agarose chips, resuspended in serum-depleted culture

medium, and transferred to Cell-Tak-coated (30 s at 42°C; BD Biosciences, Breda, The Netherlands) Ibidi microscopy culture chambers (Ibidi, Planegg, Germany). After attachment, islets were fixed in 4% phosphate-buffered paraformaldehyde for 25 min at room temperature. Fixation was followed by three 30-min washes in phosphate buffered saline (PBS) and a 3-h permeabilization in 0.3% Triton X-100/PBS. Blocking was performed by o/n incubation in 10% NGS/0.15% Triton X-100/PBS at 4°C. Islets were washed three times for 30 min with antibody diluent buffer (1% BSA/0.2 Triton X-100) before incubation with primary antibodies (guinea pig anti-insulin [Abcam, Cambridge, United Kingdom] 1:100 and rabbit antiglucagon [Vector, Peterborough, United Kingdom] 1:100, 48 h at 4°C), secondary antibodies (Alexa 647-conjugated goat anti-guinea pig [Life Technologies] and Alexa 488-conjugated goat anti-rabbit [Invitrogen], both 1:200, 48 h at 4°C), and DAPI (Invitrogen [5 mg/mL], 1:100, 20 min at room temperature). Samples were subjected to optical sectioning at 0.23-μm increments in axial (z) dimension using Nikon A1 confocal microscope (Nikon BV, Amsterdam, The Netherlands).

Glucose-induced insulin secretion test

To assess islet functionality, a glucose-induced insulin secretion test was performed at day 1 and day 5 after CI formation. Per condition per islet donor, 100 islets were used. As a control, uncoated islets were cultured in agarose chips. CI formation was performed in EGM-2, after which the islets were cultured for 5 days in islet medium, with a medium change every day. For the glucose-induced insulin secretion test, 30 islets per condition (in triplicate) were incubated for 90 min in a modified Krebs Ringer Bicarbonate (KRBH) buffer (115 mM NaCl, 5 mM KCl, 24 mM $NaHCO_3$, 2.2 mM $CaCl_2$, and 1 mM $MgCl_2$, pH 7.4), supplemented with 20 mM HEPES, 2 mg/mL human serum albumin (Sanquin, Amsterdam, The Netherlands), and 1.7 mM D-glucose.

Islets were successively incubated for 1 h in KRBH buffer with 1.7 mM and 16.7 mM D-glucose at 37°C. Insulin concentration was determined in the supernatants by ELISA (Mercodia, Uppsala, Sweden). The experiment was performed for three islet donors, each time coated with hMSCs from different donors.

Sprouting assays on Matrigel and fibrin gel

For the sprouting assays, control islets and hMSC-, HUVEC/hMSC-, and PC-hMSC-CIs were placed between two layers of growth factor-reduced Matrigel (diluted 1:1 in EGM-2; BD Biosciences) or fibrin gel (2.5 mg/mL fibrinogen and 2 μg/mL thrombin; Sigma-Aldrich). For each gel layer, 1 mL of gel was used. After polymerization, islets were cultured for 96 h in EGM-2. Islet sprouting was observed over time using an inverted microscope (Nikon Eclipse TE300). Light microscopy pictures were taken at different time points (24, 48, and 96 h) using a Nikon DS-L2 camera. The Matrigel sprouting assay was performed with islets obtained from five different human islet donors, and the fibrin sprouting assay was performed with islets from two different donors. Furthermore, hMSCs from five different donors and one clone of iMSCs were used in this study.[25] Sprout formation was manually quantified by a single person (blinded to the conditions) in minimally 10 islets per condition per time point.

Subcutaneous Matrigel plug angiogenesis assay

To test the angiogenic potential of CIs *in vivo*, control islets and hMSC-, HUVEC/hMSC-, and PC-hMSC-CIs were transplanted subcutaneously in Matrigel plugs in the back of 8-week-old male NMRI-nu mice (Harlan). For this, CIs were formed for 8 h in the agarose microwells as described before. Subsequently, islets were flushed out, washed in EGM-2 (without supplemented growth factors), and resuspended in 100 μL growth factor-reduced Matrigel (BD Biosciences). The plugs were allowed to solidify at 37°C in a round-bottom 96-well plate to obtain uniform plugs. After solidification, the Matrigel plugs were recovered from the plate and cultured for 8 h in islet culture medium. Per condition, 9 plugs were implanted containing each 200 islets. Of these plugs, three plugs were prepared using CM-DiI-prelabeled cells as described before. Before isoflurane anesthetics, mice were given 0.1 mg/kg buprenorfin (Temgesic, Schering-Plough, Belgium). Four small subcutaneous pockets were created at each quadrant of the back of the mouse. In each mouse, all four conditions were transplanted, but the position of the different conditions was randomized per animal.

Histochemistry and morphometric analysis

Two weeks after implantation, mice were sacrificed, and Matrigel plugs were recovered for histological analysis. Grafts were fixed overnight with 4% (w/v) paraformaldehyde at 4°C, embedded in paraffin blocks, and sliced into 5-μm sections.

For each Matrigel plug, sections spaced 100 μm apart were stained for insulin combined with a Masson-Goldner's trichrome staining (Merck Chemicals, Darmstadt, Germany) to determine the amount of perfused blood vessels in and around the islets. For this, primary antibody rabbit anti-insulin (1:400; Santa Cruz Biotechnology, Heidelberg, Germany) was applied for 1 h, followed by an HRP-conjugated goat anti-rabbit antibody (1:100; DAKO, Heverlee, Belgium) for 1 h. Sections were developed with DAB liquid chromogen system (DAKO) and counterstained with hematoxylin and Masson-Goldner's trichrome staining, according to the manufacturer's protocol (Merck Chemicals). Stained sections were imaged with a Nanozoomer slide scanner 2.0 RS (Hamamatsu, SZK, Japan). These sections were used to quantify the amount of perfused blood vessels in and around the islets (within 200 μm) using ImageJ software (http://rsb.info.nih.gov/ij/).

To assess the expression of insulin, glucagon, and the endothelial marker CD31, immunohistochemistry was performed on consecutive sections of islet-containing regions. For this, heat-mediated antigen retrieval (pressure cooker, 80°C, 30 min) was performed in sodium citrate buffer (pH 6.0). Sections were blocked with normal goat serum and biotin/avidin blocking kit (Vector). Primary antibodies against insulin (1:200, 1.5 h; Abcam), glucagon (1:100, o/n; Vector), and CD31 (1:20, o/n; Abcam) were used. Secondary antibodies were Alexa 647 goat anti-guinea pig (1:500, 2 h; Invitrogen), biotin-SP-conjugated anti-rabbit (1:200, 1 h; Jackson ImmunoResearch, Suffolk, United Kingdom),

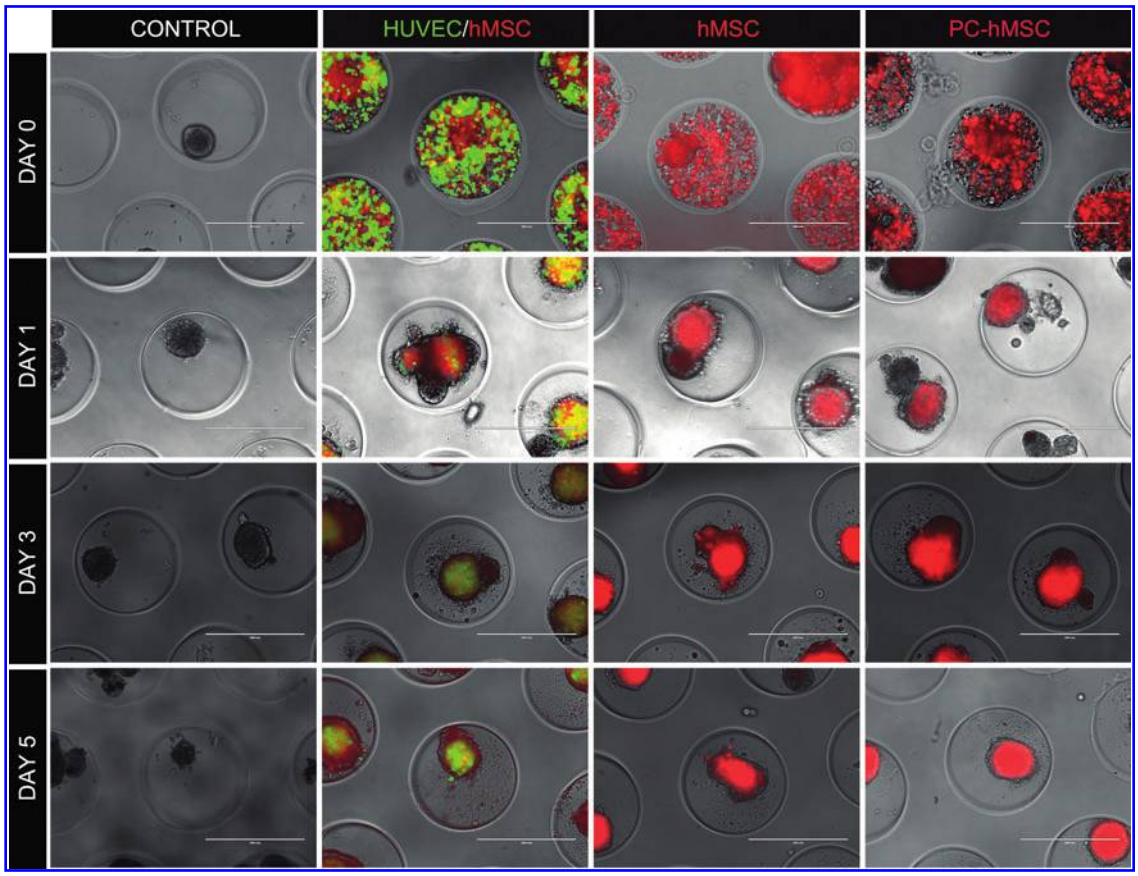

FIG. 1. Islet composite formation with different types of support cells in nonadherent agarose microwell templates. Scale bar: 400 μm. Color images available online at www.liebertpub.com/tea

and Alexa-488-SA (Invitrogen). DAPI (Invitrogen) was applied as nuclear counterstaining. Sections were imaged with a Nikon A1 confocal microscope (Nikon BV).

Statistical Analyses

Results were presented as mean ± standard error of the mean. Statistical analyses were performed using SPSS version 22 (SPSS, Chicago, IL). Kruskal–Wallis test was used for multiple group comparisons ($p < 0.05$, corrected for multiple comparisons), and paired comparisons by means of Mann–Whitney *U*-test were performed as *post hoc* test when Kruskal–Wallis test indicated significant differences.

Results

Controlled formation of CIs

To fabricate the CIs, we used a nonadherent agarose microwell culture platform, which has been developed in our group for controlled cell aggregation.[26] The microwells were seeded with single islets before seeding with fluorescently labeled single cell suspensions of HUVECs, hMSCs, or PC-hMSCs. One day after cell seeding, all single cells present in the microwells were attached to the islet. Upon culturing, the fluorescently labeled support cells became more uniformly distributed over the islets (Fig. 1). More detailed analysis of CI formation over time using 3D optical sectioning on whole-mount islets revealed that 24 h after CI formation, the cells attached to the islets as one or more cell clusters (Fig. 2A–D). During prolonged cell culture, these cell clusters migrated inward (Fig. 2E–H). Five days after initial cell seeding, all conditions, regardless of the support cell type, exhibited a core of hMSCs, HUVECs and hMSCs, or PC-hMSCs, surrounded by a mantle of insulin and glucagon-positive islet cells (Fig. 2E–H).

CIs maintain function in vitro

To assess whether the formation of CIs with different proangiogenic cell populations affects islet functionality, the insulin secretion response of the islets to a glucose challenge was measured. For all conditions, we found that stimulation with 16.7 mM glucose buffer increased the insulin secretion two to threefold compared to basal insulin secretion at 1.7 mM glucose (Fig. 2I, J) at day 1 (Fig. 2I) and day 5 (Fig. 2J). One day after CI formation, a slight, although not significant, improvement in the stimulation index was observed compared to control islets. However, this trend was not observed after 5 days of culture. At both time points, CIs and control islets showed a proper return to basal insulin secretion levels when exposed to a low, 1.7 mM, glucose buffer. These results imply that CI formation does not alter the islets' insulin secretion capacity.

CIs show increased sprout formation in vitro

In vitro angiogenesis assays were performed to assess the sprouting potential of CIs and control islets. For this, 8 h

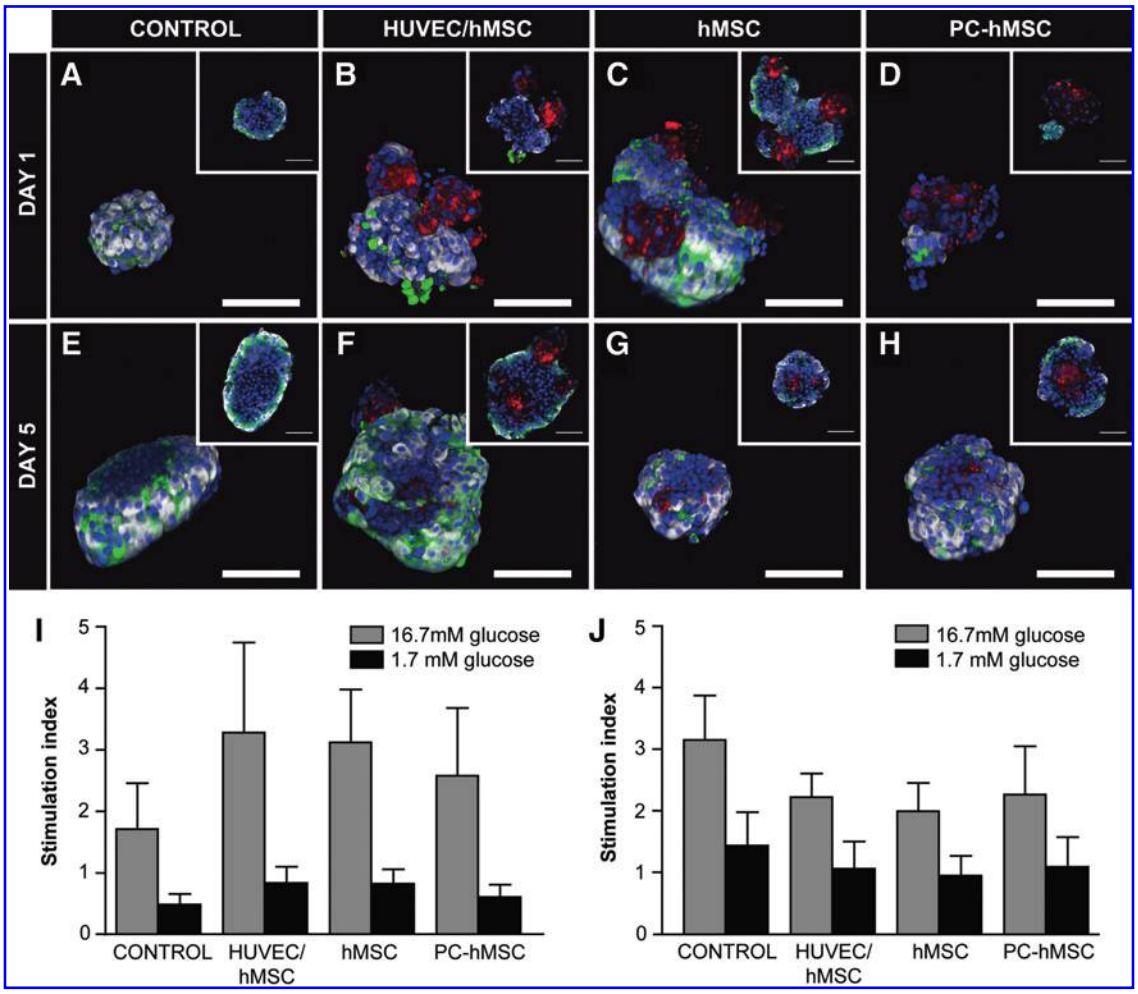

FIG. 2. Morphometric appearance and function of control islets and composite islets (CIs). **(A–H)** Representative 3D reconstructions of optically sectioned control islets and CIs at day 1 **(A–D)** and day 5 **(E–H)** after CI formation, with DiI-labeled support cells in *red*, beta-cells in *white*, and alpha-cells in *green*. The *inserts* represent an optical section through the *middle* of the islet. **(I–J)** Stimulation index of high glucose (16.7 mM) and subsequently low glucose (1.7 mM) compared to basal insulin secretion levels at 1.7 mM glucose for control islets and CIs at day 1 **(I)** and day 5 **(J)** after CI formation. Results are presented as mean ± standard error of the mean (SEM) of three independent experiments. Scale bar: 100 μm. Color images available online at www.liebertpub.com/tea

after CI formation, CIs and control islets were flushed out of the microwells and embedded in Matrigel (five different islet donors) (Fig. 3) or fibrin gel (two different islet donors) (Fig. 4). Sprouts emanating radially outward from the islets could be observed in both sprouting matrices (Fig. 3A–D and Fig. 4A–D). CM-DiI and CM-DiO labeling of the different cell types in case of HUVEC/hMSC-CIs revealed that hMSCs and HUVECs are in close contact and that both cell types contribute to sprout formation (Fig. 3E). Quantification of sprout formation in Matrigel revealed significantly more sprouts in the CI conditions compared to control islets (Fig. 3F). In particular, the number of sprouts emanating from PC-hMSC-CIs was significantly increased compared to the other conditions. This difference remained prevalent during the entire culture period, suggesting a persistent advantage of PC-hMSCs over the other observed cell types. Substituting half of the hMSCs by HUVECs did not seem to further improve sprout formation compared to hMSC-CIs. A similar trend could be observed in fibrin gel (Fig. 4E), although in contrast to Matrigel, control islets did sprout in this gel.

CIs stimulate revascularization after subcutaneous transplantation

The proangiogenic effect of CIs compared to control islets was subsequently validated using an *in vivo* Matrigel plug model. For this assay, CIs and control islets were flushed out of the microwells 8 h after cell seeding, embedded in 100 μL growth factor-reduced Matrigel, and implanted subcutaneously in the back of NMRI-nu mice. Two weeks after implantation, grafts were retrieved and prepared for histological analysis. Of the 36 implanted Matrigel plugs (9 per condition), 5 Matrigel plugs could not be recovered: 2 of the control group, 2 of the HUVEC–hMSC-CI group, and 1 of the PC-hMSC-CI group.

Insulin trichrome staining showed directed invasion of perfused vessels toward the islet grafts in case of CIs,

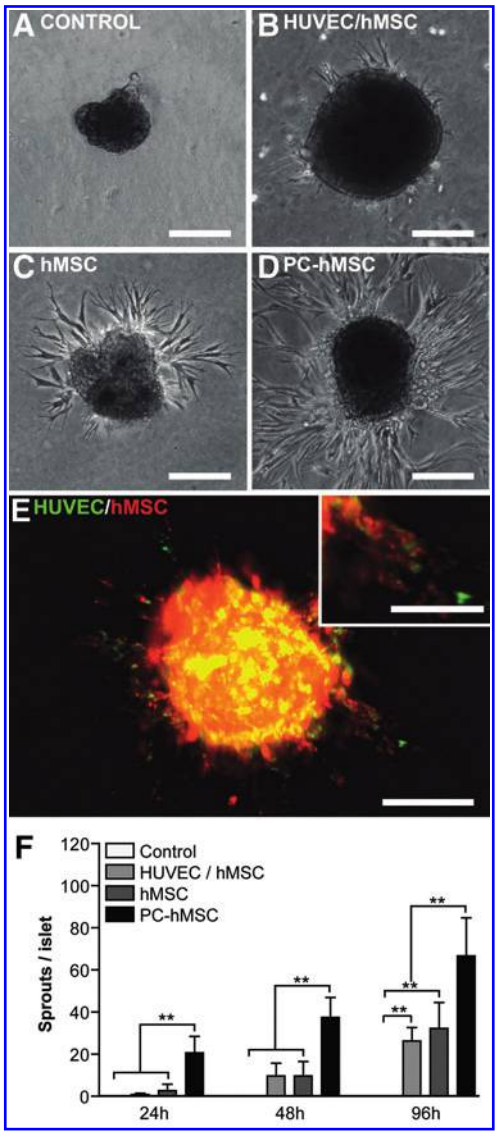

FIG. 3. Sprout formation in Matrigel. **(A–D)** Representative phase-contrast microscopy images of control islets **(A)** and CIs **(B–D)** taken 48 h after Matrigel embedding. **(E)** Representative fluorescent image of human umbilical vein endothelial cell/human mesenchymal stromal cell (HUVEC/hMSC)-CI 48 h after Matrigel embedding with HUVECs labeled with DiO (*green*) and hMSCs with DiI (*red*). Both HUVECs and hMSCs contribute to sprout formation (*insert*; scale bar: 50 μm). **(F)** Quantification of sprout formation 24, 48, and 96 h after Matrigel embedding. Results are presented as mean ± SEM of five independent experiments. ** <0.01 by Mann–Whitney *U*-test. Scale bar: 100 μm. Color images available online at www.liebertpub.com/tea

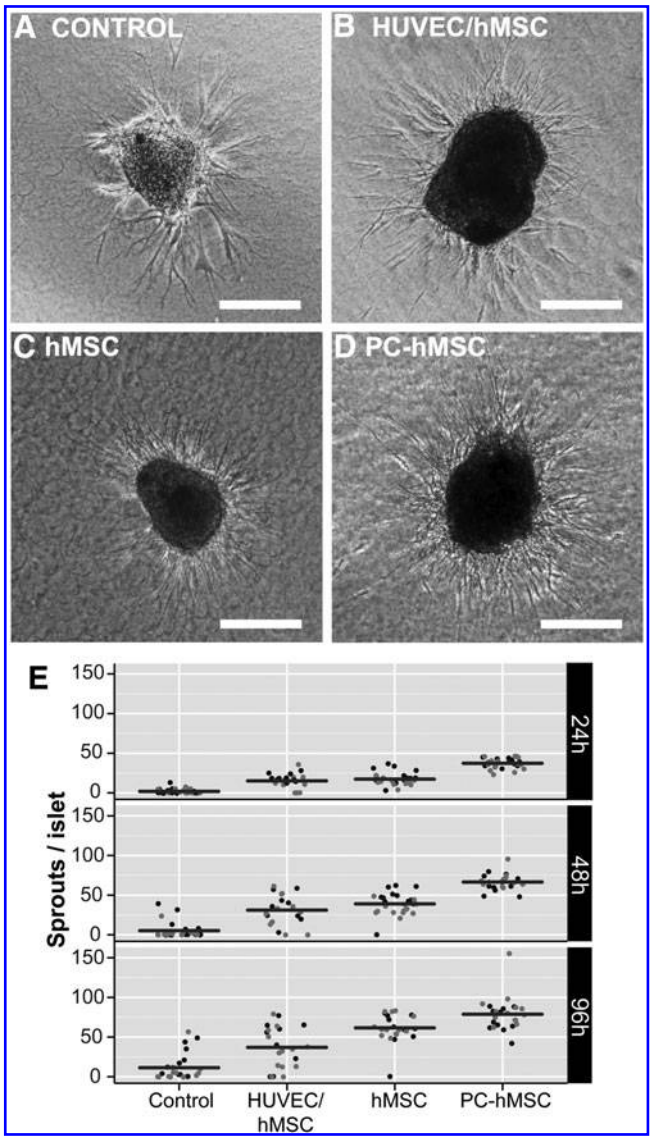

FIG. 4. Sprout formation in fibrin gel. **(A–D)** Representative phase-contrast microscopy images of control islets **(A)** and CIs **(B–D)** taken 48 h after fibrin gel embedding. **(E)** Quantification of sprout formation 24, 48, and 96 h after fibrin gel embedding. Results are presented as the mean of two independent experiments. Scale bar: 100 μm.

whereas this was limited for the control islets (Fig. 5A–D). Insulin/glucagon immunostaining of CI-containing plugs showed scattered expression patterns of both hormones throughout the islets (Fig. 5E–H), a cytoarchitecture typical for human islets.[28] In contrast to *in vitro* observations, CM-DiI labeling of CI support cells did not reveal a core–mantle structure of support cells and islet cells, respectively, when implanted *in vivo*. Further immunostaining for CD31 was performed to assess whether the CM-DiI-positive support cells expressed this endothelial marker (Fig. 5I–L). Occasionally, double-positive cells for CM-DiI and CD31 were found (Fig. 5J–L inserts), although the majority of CM-DiI-positive cells were CD31 negative.

Quantitative evaluation of vascularization in and around the islets (within 200 μm from the islet) (Fig. 6A) confirmed the observation that vascularization was increased in case of CIs compared to control islets (Fig. 6B). Only 7% of the islets in the control group were vascularized (Fig. 6C), whereas this was 32%, 19%, and 36% for HUVEC–hMSC-CIs, hMSC-CIs, and PC-hMSC-CIs, respectively (Fig. 6C). In particular, composite formation with either HUVECs combined with hMSCs or PC-hMSCs resulted in a similar increase in vascularization frequency of the islets (Fig. 6C). More detailed examination of vascular position in and around the vascularized

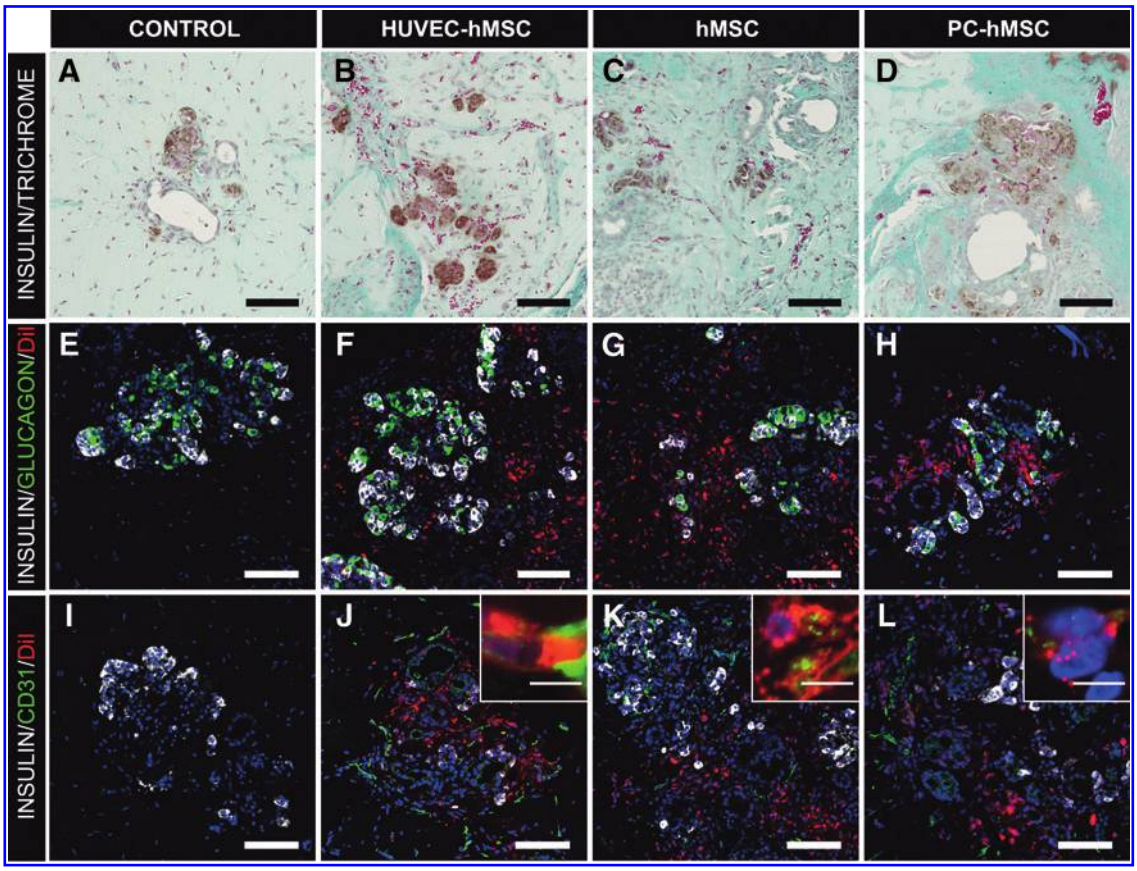

FIG. 5. Microvasculature formation and islet composition after *in vivo* subcutaneous Matrigel plug assay. **(A–D)** Representative images stained for insulin and Masson-Goldner's trichrome indicating perfused vessel formation in islet and CI grafts. **(E–L)** Representative confocal microscopy images of islets and CIs with DiI-labeled support cells, stained for insulin (*white*) and glucagon (*green*) **(E–H)** and for insulin (*white*) and CD31 (*green*) **(I–L)**. Scale bar: 100 μm. *Inserts* represent CD31-positive DiI-labeled support cells. Scale bar: 10 μm. Color images available online at www.liebertpub.com/tea

islets (Fig. 6D) revealed a general increase in the number of vessels when HUVECs were added to the composite.

Discussion

In the current study, we present a facile and reproducible method to fabricate CIs with proangiogenic cells as a strategy to improve islet vascularization after subcutaneous islet implantation. Most transplantation sites that can harbor a sufficient amount of islets, such as the subcutaneous tissue, are challenged by poor vascularization.[29] To improve the vascularization state of these sites, the possibility of creating microvascular networks using mature endothelial cells derived from vascular tissue has been suggested.[30,31] However, the limited proliferative ability of these mature endothelial cells hampers their clinical applicability. Previous studies have shown that cotransplantation of islets with either MSCs or endothelial progenitor cells can also induce neovascularization.[10–18] These cells are easier to harvest compared to mature endothelial cells and harbor greater expansion potential,[32] although a direct comparison between different support cell types for islet revascularization at a clinically relevant transplantation site has not been performed. Therefore, the study presented here directly compares the angiogenic potential of different cell types for islet revascularization in an *in vitro* and *in vivo* sprouting assay at a clinically relevant transplantation site using growth factor-reduced Matrigel as a support transplantation matrix.

Previously, we have shown that hMSCs can enhance angiogenesis in Dex-g-HA gels after subcutaneous implantation. The angiogenic potential of hMSCs could be further increased by preconditioning them in EGM-2.[24] Therefore, in this study, we evaluate the effect of hMSCs and PC-hMSCs on islet revascularization and compare their angiogenic potential with mature endothelial cells (HUVECs) supported by hMSCs, a combination shown to enhance the vascularization in subcutaneously transplanted fibrin plugs.[33]

Outlined in this study is a novel method of CI formation using a nonadherent agarose microwell platform, which allows for controlled seeding of support cells and islets in individually separated microwells. In contrast to composite formation in suspension culture, the method presented here ensures that all initially seeded cells are incorporated into the CI. In line with previous findings,[9,34,35] we observe that CI formation, regardless of the applied support cell type, does not affect the glucose-stimulated insulin secretion capacity of the islets. The first day after CI formation, the stimulation indices of CIs are slightly, although not significantly, higher compared to those of control islets. However, this trend is not observed 5 days after coculture. This initial

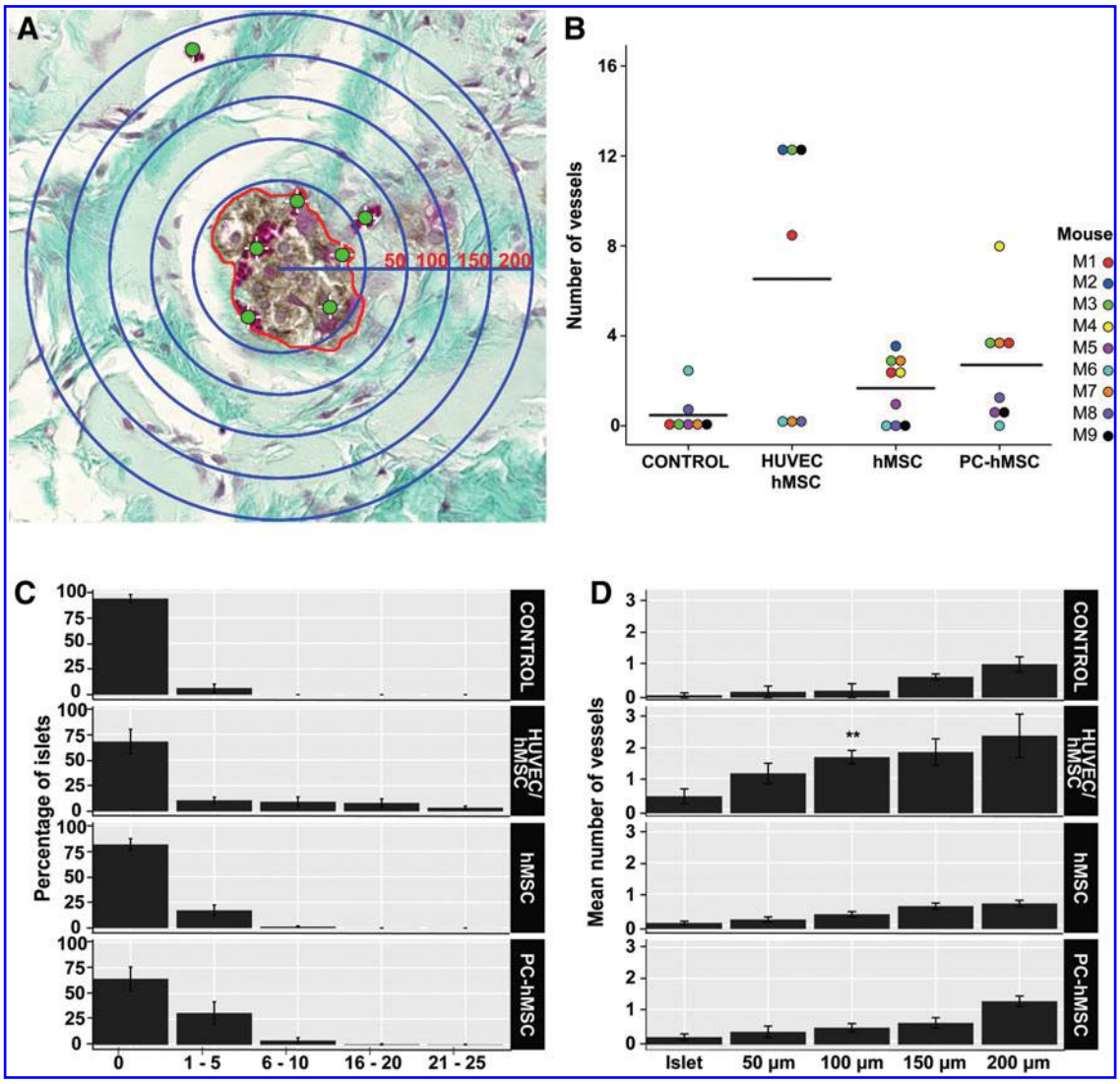

FIG. 6. Vascular morphometry of control islets and CIs. **(A)** Image segmentation to determine the number of blood vessels in the insulin-positive islet area (*red*) and within a radius of 200 µm (divided into 50-µm sections as represented in *blue*). **(B)** Number of blood vessels per islet for uncoated control islets and CIs. **(C)** Frequency plot for vascularization of control islets and CIs. **(D)** Mean number of vessels within vascularized islets. Results are presented as mean ± SEM. **<0.01 by Mann–Whitney *U*-test. Scale bar: 100 µm. Color images available online at www.liebertpub.com/tea

boost in the insulin secretion profile has been reported previously by Dubiel *et al.*, who cocultured porcine islets on a monolayer of porcine liver microvascular endothelial cells.[36] They showed that the stimulated insulin secretion improved for 2 days but not for more than 7 days. Similar to Dubiel *et al.*, we cultured the islets in islet culture medium, which might not optimally support the function of the support cells. This might explain why we observe an initial supportive effect, which is lost over time.

The cellular organization of CIs is dramatically changed during *in vitro* culture when no supporting matrix, such as Matrigel or fibrin gel, is used. We show that the support cells preferentially aggregate before adhering to the islets, an observation in line with previous reports.[9,35] These aggregates are engulfed over time by the islet cell mass, corroborating earlier observations by Johansson *et al.*, who described similar internalization of endothelial cells upon culturing.[9] Such phenomena can be explained by the ''differential adhesion hypothesis''; this postulates that a population of motile mutually adhesive cells will spontaneously reorganize to substitute weaker intercellular adhesions for stronger ones until a configuration is found that maximizes adhesive bonding.[37] This results in a core of cells with strong cohesive interactions surrounded by a shell of cells exhibiting lower affinity. Our observation that the islet cells envelop the support cells suggests that islet cells are less cohesive compared to the support cells. This may be partly attributed to the enzymatic procedure for islet isolation, which destroys the islets' basement membrane and alters the cellular integrin expression.[38] However, we observe an opposite pattern when CIs are embedded in Matrigel or fibrin gel after 8 h of CI formation, with extensive sprouting of the support cells radially outward to form an outer CI layer penetrating the surrounding gel. In addition, after *in vivo* implantation of CIs in supporting Matrigel plugs, intact islet cell clusters are observed surrounded by outwardly migrating support cells.

These observations suggest that sufficient cell–matrix interactions can counteract the cell–cell-based differential adhesion phenomenon observed when no matrix is used.

Established *in vitro* models of angiogenesis involve the monitoring of capillary-like structure formation in three-dimensional gels mimicking the extracellular matrix, such as Matrigel,[39] fibrin gel,[9,40,41] or collagen gel.[42] Using two of these model systems, Matrigel and fibrin gel, we demonstrate the induction of sprout formation of CIs over time. The observation that control islets sprout in fibrin gel is in line with previous reports.[9,36,41,43] To our knowledge, no studies have monitored sprouting of control islets in Matrigel. The improved performance in the sprouting capacity of uncoated islets in fibrin compared to Matrigel could be explained by either the difference in matrix composition or stiffness, shown to influence sprout formation of mature endothelial cells both *in vitro*[42,44–47] and *in vivo*,[33] or the expression of a specific set of fibrinolytic enzymes in intraislet endothelial cells, specifically enabling successful remodeling of the fibrin matrix.[47–49] Our results further indicate that hMSCs, either naive or preconditioned, significantly enhance sprout formation *in vitro* compared to HUVEC–hMSC-CIs or control islets. Cellular invasion and migration in Matrigel, which primarily consists of laminin, collagen IV, and enactin, are mainly regulated by matrix metalloprotease (MMP) activity.[50] It has been shown that bone marrow-derived hMSCs express higher levels of MMPs, such as MT1-MMP and MMP-2, compared to HUVECs.[40] The twofold reduction of hMSCs in the HUVEC–hMSC-CI group compared to the hMSC- and PC-hMSC-CI group likely results in a lower expression of proteolytic enzymes, accounting for the observed reduction in *in vitro* sprout formation.

Analysis of *in vivo* implanted Matrigel plugs reveals the highest number of perfused lumen-containing structures in and around the islets when islets are coimplanted with a mixture of HUVECs and hMSCs. Although contrary to our *in vitro* findings, this observation is in line with a previous report, where subcutaneously transplanted fibrin plugs containing a mixture of hMSCs and HUVECs exhibited an increased number of perfused vessels compared to constructs containing either hMSCs or HUVECs alone.[47] The exact mechanisms by which the hMSC–HUVEC combination results in improved neogenesis remain unclear. However, hMSCs and HUVECs secrete numerous soluble factors, such as vascular endothelial growth factor and bFGF,[51] and extracellular matrix (ECM)-remodeling enzymes, such as MMPs.[52] All these factors contribute to endothelial cell survival, tip cell migration, proliferation, endothelial tubulogenesis, and/or vascular maturation,[52–54] and many of them have been shown to have increased expression in HUVEC/HMSC cocultures.[40] Others suggest that hMSCs are able to differentiate into a pericyte or smooth muscle-like cell and stabilize newly formed vessels,[55] but further studies are warranted.

Although an increasing trend is observed in the percentage of vascularized islets when hMSCs or PC-hMSCs are used compared to control islets, we do not observe a significant difference in perfused vessel density. This is in contrast to previous studies reporting a significant improvement in revascularization rate when islets are cotransplanted with bone marrow-derived MSCs underneath the renal capsule[11,13] or intrahepatically.[12] The reason for this discrepancy is likely multifactorial. First of all, since the subcutaneous site is poorly vascularized compared to the renal subcapsular and intrahepatic transplantation sites, the proangiogenic effect of hMSCs might be unable to induce islet revascularization at this transplantation site within the observed period. Furthermore, growth factor-reduced Matrigel as a supporting matrix imposes an additional barrier for both the diffusion of soluble proangiogenic factors and the infiltration of blood vessels. Another factor that could have influenced the revascularization process is the distribution of the support cells within the hydrogel. In the current study, the support cells are confined around the islets. However, we have previously shown that when hMSCs and PC-hMSCs are homogeneously distributed throughout Dex-g-HA gels, these cells can significantly improve the vascularization in these gels.[24] Although Dex-g-HA was used in lieu of Matrigel, possibly accounting for the difference in outcome, we hypothesize that the uniform cellular distribution created a more suitable environment for recruitment and infiltration of blood vessels because of a more homogeneous presentation of angiogenic and proteolytic factors.

Comparing the *in vitro* and *in vivo* results, we observe a discrepancy in angiogenesis. *In vivo*, the highest number of perfused lumen in and around the islets is observed when islets are coimplanted with a mixture of HUVECs and hMSCs, whereas *in vitro* sprout formation is highest for the PC-hMSC-CIs. As mentioned before, *in vitro* tube formation is largely influenced by the invasive capacity of the cells by proteolytic enzyme-mediated ECM degradation. Although ECM degradation is an important component for *in vivo* angiogenesis, the process of *de novo* blood vessel formation involves a complex interplay between multiple cell types from host and donor, growth factors, enzymes, and extracellular matrix molecules, a level of complexity not mimicked during *in vitro* assays and therefore precludes an accurate prediction of *in vivo* outcome. Our findings highlight the importance and necessity of verifying *in vitro* studies with *in vivo* models to reliably predict, in this case, revascularization outcomes.

In conclusion, this study presents a controlled method to fabricate CIs to study the angiogenic potential of different support cells. Using a subcutaneous Matrigel plug assay, we demonstrate that CIs with hMSCs and HUVECs exhibit a higher angiogenic potential compared to control islets or islets combined with hMSCs or PC-hMSCs. However, for this method to be clinically applicable, further efforts are required to identify a clinically relevant proangiogenic cell source. Regardless, we show here the therapeutic potential of CIs with proangiogenic support cells to enhance islet revascularization at a clinically relevant, although poorly vascularized, transplantation site.

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Disclosure Statement

No competing financial interests exist.

References

1. Ryan, E.A., Paty, B.W., Senior, P.A., Bigam, D., Alfadhli, E., Kneteman, N.M., *et al.* Five-year follow-up after clinical islet transplantation. Diabetes. 2005/06/29 ed. **54,** 2060, 2005.
2. Shapiro, A.M., Lakey, J.R., Ryan, E.A., Korbutt, G.S., Toth, E., Warnock, G.L., *et al.* Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med **343,** 230, 2000.
3. Barton, F.B., Rickels, M.R., Alejandro, R., Hering, B.J., Wease, S., Naziruddin, B., *et al.* Improvement in outcomes of clinical islet transplantation: 1999–2010. Diabetes Care **35,** 1436, 2012.
4. Desai, N.M., Goss, J.A., Deng, S., Wolf, B.A., Markmann, E., Palanjian, M., *et al.* Elevated portal vein drug levels of sirolimus and tacrolimus in islet transplant recipients: local immunosuppression or islet toxicity? Transplantation **76,** 1623, 2003.
5. Moberg, L., Johansson, H., Lukinius, A., Berne, C., Foss, A., Källen, R., *et al.* Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. Lancet **360**, 11, 2002.
6. Johansson, H., Lukinius, A., Moberg, L., Lundgren, T., Berne, C., Foss, A., *et al.* Tissue factor produced by the endocrine cells of the islets of Langerhans is associated with a negative outcome of clinical islet transplantation. Diabetes **54,** 1755, 2005.
7. Sakata, N., Aoki, T., Yoshimatsu, G., Tsuchiya, H., Hata, T., Katayose, Y., *et al.* Strategy for clinical setting in intramuscular and subcutaneous islet transplantation. Diabetes Metab Res Rev **30,** 1, 2014.
8. Martino, M.M., Brkic, S., Bovo, E., Burger, M., Schäfer, D.J., Wolff, T., *et al.* Tissue engineering and regenerative medicine extracellular matrix and growth factor engineering for controlled angiogenesis in regenerative medicine. Front Bioeng Biotechnol **3,** 45, 2015.
9. Johansson, U., Rasmusson, I., Niclou, S.P., Forslund, N., Gustavsson, L., Nilsson, B., *et al.* Formation of composite endothelial cell-mesenchymal stem cell islets: a novel approach to promote islet revascularization. Diabetes **57,** 2393, 2008.
10. Li, Y., Xue, W., Liu, H., Fan, P., Wang, X., Ding, X., *et al.* Combined strategy of endothelial cells coating, sertoli cells coculture and infusion improves vascularization and rejection protection of islet graft. PLoS One **8,** 2013.
11. Figliuzzi, M., Cornolti, R., Perico, N., Rota, C., Morigi, M., Remuzzi, G., *et al.* Bone marrow-derived mesenchymal stem cells improve islet graft function in diabetic rats. Transplant Proc **41,** 1797, 2009.
12. Ito, T., Itakura, S., Todorov, I., Rawson, J., Asari, S., Shintaku, J., *et al.* Mesenchymal stem cell and Islet cotransplantation promotes graft revascularization and function. Transplantation **89,** 1438, 2010.
13. Sakata, N., Chan, N.K., Chrisler, J., Obenaus, A., and Hathout, E. Bone marrow cell cotransplantation with islets improves their vascularization and function. Transplantation **89,** 686, 2010.
14. Rackham, C.L., Chagastelles, P.C., Nardi, N.B., Hauge-Evans, A.C., Jones, P.M., and King, A.J.F. Co-transplantation of mesenchymal stem cells maintains islet organisation and morphology in mice. Diabetologia **54,** 1127, 2011.
15. Cavallari, G., Olivi, E., Bianchi, F., Neri, F., Foroni, L., Valente, S., *et al.* Mesenchymal stem cells and islet co-transplantation in diabetic rats: improved islet graft revascularization and function by human adipose tissue-derived stem cells preconditioned with natural molecules. Cell Transplant **21,** 2771, 2012.
16. Oh, B.J., Oh, S.H., Jin, S.M., Suh, S., Bae, J.C., Park, C.-G., *et al.* Co-transplantation of bone marrow-derived endothelial progenitor cells improves revascularization and organization in islet grafts. Am J Transplant **13,** 1429, 2013.
17. Quaranta, P., Antonini, S., Spiga, S., Mazzanti, B., Curcio, M., Mulas, G., *et al.* Co-transplantation of endothelial progenitor cells and pancreatic islets to induce long-lasting normoglycemia in streptozotocin-treated diabetic rats. PLoS One **9,** e94783, 2014.
18. Kang, S., Park, H.S., Jo, A., Hong, S.H., Lee, H.N., Lee, Y.Y., *et al.* Endothelial progenitor cell cotransplantation enhances islet engraftment by rapid revascularization. Diabetes **61,** 866, 2012.
19. Sakata, N., Tan, A., Chan, N.K., Obenaus, A., Mace, J., Peverini, R., *et al.* ITSC08 efficacy comparison between intraportal and subcapsular islet transplants in a murine diabetic model. Transplant Proc **41,** 346, 2010.
20. Zhang, P., Moudgill, N., Hager, E., Tarola, N., Dimatteo, C., McIlhenny, S., *et al.* Endothelial differentiation of adipose-derived stem cells from elderly patients with cardiovascular disease. Stem Cells Dev **20,** 977, 2011.
21. Wang, H. Shear stress induces endothelial differentiation from a murine embryonic mesenchymal progenitor cell line. Arterioscler Thromb Vasc Biol **25,** 1817, 2005.
22. Fischer, L.J., McIlhenny, S., Tulenko, T., Golesorkhi, N., Zhang, P., Larson, R., *et al.* Endothelial differentiation of adipose-derived stem cells: effects of endothelial cell growth supplement and shear force. J Surg Res **152,** 157, 2009.
23. Bai, K., Huang, Y., Jia, X., Fan, Y., and Wang, W. Endothelium oriented differentiation of bone marrow mesenchymal stem cells under chemical and mechanical stimulations. J Biomech **43,** 1176, 2010.
24. Portalska, K.J., Teixeira, L.M., Leijten, J.C.H., Jin, R., van Blitterswijk, C., de Boer, J., *et al.* Boosting angiogenesis and functional vascularization in injectable Dextran–Hyaluronic acid hydrogels by endothelial-like mesenchymal stromal cells. Tissue Eng Part A **20,** 819–829, 2013.
25. Skårn, M., Noordhuis, P., Wang, M.-Y., Veuger, M., Henrichson Kresse, S., Egeland, E.V., *et al.* Generation and characterization of an immortalized human mesenchymal stromal cell line. Stem Cells Dev **23,** 2377, 2014.
26. Rivron, N.C., Vrij, E.J., Rouwkema, J., Le Gac, S., van den Berg, A., Truckenmuller, R.K., *et al.* Tissue deformation spatially modulates VEGF signaling and angiogenesis. Proc Natl Acad Sci **109,** 6886, 2012.
27. Hilderink, J., Spijker, S., Carlotti, F., Lange, L., Engelse, M., van Blitterswijk, C., *et al.* Controlled aggregation of primary human pancreatic islet cells leads to glucose-responsive pseudoislets comparable to native islets. J Cell Mol Med **19,** 1836, 2015.
28. Cabrera, O., Berman, D.M., Kenyon, N.S., Ricordi, C., Berggren, P., and Caicedo, A. The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. Proc Natl Acad Sci **103,** 2334, 2006.

29. Kemp, C.B., Knight, M.J., Scharp, D.W., Ballinger, W.F., and Lacy, P.E. Effect of transplantation site on the results of pancreatic islet isografts in diabetic rats. Diabetologia **9,** 486, 1973.
30. Schechner, J.S., Nath, A.K., Zheng, L., Kluger, M.S., Hughes, C.C., Sierra-Honigmann, M.R., *et al.* In vivo formation of complex microvessels lined by human endothelial cells in an immunodeficient mouse. Proc Natl Acad Sci U S A **97,** 9191, 2000.
31. Nör, J.E., Peters, M.C., Christensen, J.B., Sutorik, M.M., Linn, S., Khan, M.K., *et al.* Engineering and characterization of functional human microvessels in immunodeficient mice. Lab Invest **81,** 453, 2001.
32. Bara, J.J., Richards, R.G., Alini, M., and Stoddart, M.J. Concise review: bone marrow-derived mesenchymal stem cells change phenotype following in vitro culture: implications for basic research and the clinic. Stem Cells **32,** 1713, 2014.
33. Kniazeva, E., Kachgal, S., and Putnam, A.J. Effects of extracellular matrix density and mesenchymal stem cells on neovascularization in vivo. Tissue Eng Part A **17,** 905, 2011.
34. Rackham, C.L., Dhadda, P.K., Chagastelles, P.C., Simpson, S.J.S., Dattani, A.A., Bowe, J.E., *et al.* Pre-culturing islets with mesenchymal stromal cells using a direct contact configuration is beneficial for transplantation outcome in diabetic mice. Cytotherapy **15,** 449, 2013.
35. Jung, H.S., Kim, M.J., Hong, S.H., Lee, Y.J., Kang, S., Lee, H., *et al.* The potential of endothelial colony-forming cells to improve early graft loss after intraportal islet transplantation. Cell Transplant **23,** 273, 2014.
36. Dubiel, E.A., Lakey, J.R.T., Lamb, M.W., and Vermette, P. Culturing free-floating and fibrin-embedded islets with endothelial cells: effects on insulin secretion and apoptosis. Cell Mol Bioeng **7,** 243, 2014.
37. Foty, R.A., and Steinberg, M.S. The differential adhesion hypothesis: a direct evaluation. Dev Biol **278,** 255, 2005.
38. Wang, R.N., Paraskevas, S., and Rosenberg, L. Characterization of integrin expression in islets isolated from hamster, canine, porcine, and human pancreas. J Histochem Cytochem **47,** 499, 1999.
39. Donovan, D., Brown, N.J., Bishop, E.T., and Lewis, C.E. Comparison of three in vitro human ''angiogenesis'' assays with capillaries formed in vivo. Angiogenesis **4,** 113, 2001.
40. Ghajar, C.M., Kachgal, S., Kniazeva, E., Mori, H., Costes, S.V., George, S.C., *et al.* Mesenchymal cells stimulate capillary morphogenesis via distinct proteolytic mechanisms. Exp Cell Res **316**, 813, 2010.
41. Kidszun, A., Schneider, D., Erb, D., Hertl, G., Schmidt, V., Eckhard, M., *et al.* Isolated pancreatic islets in three-dimensional matrices are responsive to stimulators and inhibitors of angiogenesis. Cell Transplant **15,** 489, 2006.
42. Abbey, C.A., and Bayless, K.J. Matrix density alters zyxin phosphorylation, which limits peripheral process formation and extension in endothelial cells invading 3D collagen matrices. Matrix Biol **38,** 36, 2014.
43. Linn, T., Schneider, K., Hammes, H.P., Preissner, K.T., Brandhorst, H., Morgenstern, E., *et al.* Angiogenic capacity of endothelial cells in islets of Langerhans. FASEB J **17,** 881, 2003.
44. Sieminski, A.L, Hebbel, R.P., and Gooch, K.J. The relative magnitudes of endothelial force generation and matrix stiffness modulate capillary morphogenesis in vitro. Exp Cell Res **297,** 574, 2004.
45. Sieminski, A.L., Was, A.S., Kim, G., Gong, H., and Kamm, R.D. The stiffness of three-dimensional ionic self-assembling peptide gels affects the extent of capillary-like network formation. Cell Biochem Biophys **49,** 73, 2007.
46. Ghajar, C.M., Chen, X., Harris, J.W., Suresh, V., Hughes, C.C.W., Jeon, N.L., *et al.* The effect of matrix density on the regulation of 3-D capillary morphogenesis. Biophys J **94,** 1930, 2008.
47. Kniazeva, E., and Putnam, A.J. Endothelial cell traction and ECM density influence both capillary morphogenesis and maintenance in 3-D. Am J Physiol Cell Physiol **297,** C179, 2009.
48. Hiraoka, N., Allen, E., Apel, I.J., Gyetko, M.R., and Weiss, S.J. Matrix metalloproteinases regulate neovascularization by acting as pericellular fibrinolysins. Cell **95,** 365, 1998.
49. Kim, I., Kim, H.G., Moon, S.O., Chae, S.W., So, J.N., Koh, K.N., *et al.* Angiopoietin-1 induces endothelial cell sprouting through the activation of focal adhesion kinase and plasmin secretion. Circ Res **86,** 952, 2000.
50. Taraboletti, G., D'Ascenzo, S., Borsotti, P., Giavazzi, R., Pavan, A., and Dolo, V. Shedding of the matrix metalloproteinases MMP-2, MMP-9, and MT1-MMP as membrane vesicle-associated components by endothelial cells. Am J Pathol **160,** 673, 2002.
51. da Silva Meirelles, L., Fontes, A.M., Covas, D.T., and Caplan, A.I. Mechanisms involved in the therapeutic properties of mesenchymal stem cells. Cytokine Growth Factor Rev **20,** 419, 2009.
52. Chen, Q., Jin, M., Yang, F., Zhu, J., Xiao, Q., and Zhang, L. Matrix metalloproteinases: inflammatory regulators of cell behaviors in vascular formation and remodeling. Mediators Inflamm **2013,** 2013, 14.
53. Carmeliet, P. Mechanisms of angiogenesis and arteriogenesis. Nat Med **6,** 389, 2000.
54. Cross, M.J., and Claesson-Welsh, L. FGF and VEGF function in angiogenesis: signalling pathways, biological responses and therapeutic inhibition. Trends Pharmacol Sci **22**, 201, 2001.
55. Grainger, S.J., Carrion, B., Ceccarelli, J., and Putnam, A.J. Stromal cell identity influences the in vivo functionality of engineered capillary networks formed by co-delivery of endothelial cells and stromal cells. Tissue Eng Part A **19,** 1209, 2013.

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2. M. Birgul Akolpoglu, Yasemin Inceoglu, Ugur Bozuyuk, Ana Rita Sousa, Mariana B. Oliveira, João F. Mano, Seda Kizilel. 2021. Recent advances in the design of implantable insulin secreting heterocellular islet organoids. *Biomaterials* **269**, 120627. [Crossref]
3. Elisa Maillard. Plasma scaffolds for islet transplantation 257-268. [Crossref]
4. Olle Korsgren, Hanne Scholz. Cellular therapies in preclinical and clinical islet transplantation: Mesenchymal stem cells 821-831. [Crossref]
5. Elisa Maillard, Séverine Sigrist. From insulin replacement to bioengineered, encapsulated organoids 299-333. [Crossref]
6. Heide Brandhorst, Daniel Brandhorst, Anju Abraham, Samuel Acreman, Simen W. Schive, Hanne Scholz, Paul R.V. Johnson. 2020. Proteomic Profiling Reveals the Ambivalent Character of the Mesenchymal Stem Cell Secretome: Assessing the Effect of Preconditioned Media on Isolated Human Islets. *Cell Transplantation* **29**, 096368972095233. [Crossref]
7. Irit Meivar-Levy, Fatima Zoabi, Gil Nardini, Eugenia Manevitz-Mendelson, Gil S. Leichner, Oranit Zadok, Michael Gurevich, Eytan Mor, Simona Dima, Irinel Popescu, Aviv Barzilai, Sarah Ferber, Shoshana Greenberger. 2019. The role of the vasculature niche on insulin-producing cells generated by transdifferentiation of adult human liver cells. *Stem Cell Research & Therapy* **10**:1. . [Crossref]
8. Shuixan Hu, Paul de Vos. 2019. Polymeric Approaches to Reduce Tissue Responses Against Devices Applied for Islet-Cell Encapsulation. *Frontiers in Bioengineering and Biotechnology* **7**. . [Crossref]
9. Vivian Lehmann, Parker L. Andersen, Rajesh G. Damodaran, Patrick Vermette. 2019. Method for isolation of pancreatic blood vessels, their culture and coculture with islets of langerhans. *Biotechnology Progress* **35**:2, e2745. [Crossref]
10. Hadavi Elahe, Leijten Jeroen, Engelse Marten, de Koning Eelco, Jonkheijm Pascal, Karperien Marcel, van Apeldoorn Aart. 2019. Microwell Scaffolds Using Collagen-IV and Laminin-111 Lead to Improved Insulin Secretion of Human Islets. *Tissue Engineering Part C: Methods* **25**:2, 71-81. [Abstract] [Full Text] [PDF] [PDF Plus] [Supplementary Material]
11. Dr. Elahe Hadavi, Prof. Jeroen C.H. Leijten, Dr. Marten A. Engelse, Prof. Eelco de Koning, Dr. Pascal Jonkheijm, Dr. Marcel Karperien, Dr. Aart Alexander van Apeldoorn. Microwell scaffolds using collagen-IV and laminin-111 lead to improved insulin secretion of islets. *Tissue Engineering Part C: Methods* **0**:ja. . [Abstract] [PDF] [PDF Plus]
12. Marcos Perez-Basterrechea, Manuel M. Esteban, Jose A. Vega, Alvaro J. Obaya. 2018. Tissue-engineering approaches in pancreatic islet transplantation. *Biotechnology and Bioengineering* **115**:12, 3009-3029. [Crossref]
13. Milou Groot Nibbelink, Katarzyna Skrzypek, Lisanne Karbaat, Sanne Both, Jacqueline Plass, Bettie Klomphaar, Jéré van Lente, Sieger Henke, Marcel Karperien, Dimitrios Stamatialis, Aart van Apeldoorn. 2018. An important step towards a prevascularized islet microencapsulation device: in vivo prevascularization by combination of mesenchymal stem cells on micropatterned membranes. *Journal of Materials Science: Materials in Medicine* **29**:11. . [Crossref]
14. Alexander Ulrich Ernst, Long-Hai Wang, Minglin Ma. 2018. Islet encapsulation. *Journal of Materials Chemistry B* **6**:42, 6705-6722. [Crossref]
15. Gwang-Bum Im, Suk Ho Bhang. 2018. Recent research trend in cell and drug delivery system for type 1 diabetes treatment. *Journal of Pharmaceutical Investigation* **48**:2, 175-185. [Crossref]
16. Jung-Sik Kim, Hyunwoo Chung, Nari Byun, Seong-Jun Kang, Sunho Lee, Jun-Seop Shin, Chung-Gyu Park. 2018. Construction of EMSC-islet co-localizing composites for xenogeneic porcine islet transplantation. *Biochemical and Biophysical Research Communications* **497**:2, 506-512. [Crossref]
17. Katarzyna Skrzypek, Yazmin Brito Barrera, Thomas Groth, Dimitrios Stamatialis. 2018. Endothelial and beta cell composite aggregates for improved function of a bioartificial pancreas encapsulation device. *The International Journal of Artificial Organs* **41**:3, 152-159. [Crossref]
18. Alexandra M. Smink, Shiri Li, Daniël H. Swart, Don T. Hertsig, Bart J. de Haan, Jan A. A. M. Kamps, Leendert Schwab, Aart A. van Apeldoorn, Eelco de Koning, Marijke M. Faas, Jonathan R. T. Lakey, Paul de Vos. 2017. Stimulation of vascularization of a subcutaneous scaffold applicable for pancreatic islet-transplantation enhances immediate post-transplant islet graft function but not long-term normoglycemia. *Journal of Biomedical Materials Research Part A* **105**:9, 2533-2542. [Crossref]
19. Alexander E. Vlahos, Nicholas Cober, Michael V. Sefton. 2017. Modular tissue engineering for the vascularization of subcutaneously transplanted pancreatic islets. *Proceedings of the National Academy of Sciences* **114**:35, 9337-9342. [Crossref]

20. Guang-Zhen Jin, Hae-Won Kim. 2017. Co-culture of Human Dental Pulp Stem Cells and Endothelial Cells Using Porous Biopolymer Microcarriers: A Feasibility Study for Bone Tissue Engineering. *Tissue Engineering and Regenerative Medicine* **14**:4, 393-401. [Crossref]
21. Ruimin Long, Yuangang Liu, Shibin Wang, Li Ye, Peng He. 2017. Co-Microencapsulation of BMSCs and Mouse Pancreatic (3 Cells for Improving the Efficacy of Type I Diabetes Therapy. *The International Journal of Artificial Organs* **40**:4, 169-175. [Crossref]
22. Omaima M. Sabek. Advancing Islet Transplantation: From Donor to Engraftment 189-211. [Crossref]
23. Luba Perry, Shahar Ben-Shaul, Shira Landau, Shulamit Levenberg. Co-Culture Systems for Vasculogenesis 1-29. [Crossref]
24. Afonso Malheiro, Paul Wieringa, Carlos Mota, Matthew Baker, Lorenzo Moroni. 2016. Patterning Vasculature: The Role of Biofabrication to Achieve an Integrated Multicellular Ecosystem. *ACS Biomaterials Science & Engineering* **2**:10, 1694-1709. [Crossref]